the source of myosin? (3) Does the IFM-specific troponin keep the myosin from generating force prematurely? To answer these questions we have started experiments to exchange contractile proteins within IFM sarcomere with those from other sources. It is shown that, when IFM fibers from crane fly are incubated in a solution containing fluorescent rabbit skeletal muscle myosin, it is incorporated into IFM sarcomere. This technique utilizes the properties of myosins that have different extractability in KCl solutions. The IFM fibers with rabbit myosin showed a series of strong X-ray meridional reflections with a basic repeat of 14.5 nm. On the other hand, the control IFM fibers incubated in the same KCl solution without rabbit myosin seem to retain their endogenous myosin molecules. These results suggest that, even at KCl concentrations too low to extract endogenous myosin, myosin exchange occurs if exogenous solubilized myosin is present in the surrounding medium. We also plan to extract the thin filaments in IFM and to replace them with actin filaments from other sources.

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The M-Line Protein Obscurin in the Development of Insect Flight Muscle

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The Drosophila M-line protein, obscurin (475 kD) has 21 tandem Ig domains, 3 Fn3 domains, a Rho-GEF signalling domain near the N-terminus and two kinase domains near the C-terminus. Obscurin is needed for the formation of a symmetrical sarcomere in the indirect flight muscle (IFM). Obscurin forms periodic striations in the IFM sarcomere 30 h after puparium formation, when kettin and myosin are still in unstructured strands and there are no Z-discs. Early expression of obscurin acts as a template for the formation of symmetrical thick filaments. Reducing expression by RNAi had no effect on sarcomere length in IFM, but the M-line was missing and the H-zone was shifted from the midline of the sarcomere. The length and polarity of thin filaments was determined by the position of the bare zone in adjacent thick filaments. Therefore obscurin is essential for the assembly of correctly overlapped thick and thin filaments in a symmetrical sarcomere. We have identified ligands of the two kinase domains in vivo. Kinase constructs with tags were injected into embryos and individual kinases with bound proteins were isolated from IFM. Kinase 1 bound Ball (an active kinase) and kinase 2 bound MASK (a 400 kD protein with ankyrin repeats). Reducing expression of these proteins by RNAi produced a phenotype in IFM that was similar to that produced by reducing obscurin, although obscurin itself was present at wild-type levels. Therefore Ball and MASK probably act downstream of obscurin. These proteins are linked via signalling pathways involved in the development of Drosophila muscle.

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Sarcolemmal Biomechanics and Excitability in Malformed Muscle Fibers of Dystrophic Mice

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Background: Duchenne muscular dystrophy (DMD), the most common and severe dystrophy, is caused by the absence of dystrophin. Muscle weakness and fragility (i.e. increased susceptibility to damage) are presumably due to structural weakness of the myofiber cytoskeleton, but recent studies suggest that malformed/split myofibers in dystrophic muscle may also play a role. We have previously studied the biomechanical properties of the sarcolemma in single myofibers isolated mechanically from extensor digitorum longus (EDL) muscles in wild-type (WT) and dystrophic (mdx, mouse model for DMD) mice. PURPOSE: We use similar biomechanical methods on enzymatically-dissociated myofibers (both normal and malformed) from the flexor digitorum brevis muscle (FDB) of WT and mdx mice.
Methods: FDB muscles were enzymatically-dissociated and plated on specialized coverslips. Suction pressures (P) applied through a pipette to the membrane generated a bleb, which increased in height with increasing P. Larger increases in P ruptured the connections between the sarcolemma and myofibrils (speration P, or SP) and eventually caused the sarcolemma to burst. We also examined excitability using high-speed confocal microscopy and the voltage-sensitive indicator di-8-butyl-amino-naphthyl-ethylene-pyridinium-propyl-sulfonate to assess the action potential (AP).
Results: The mechanical results from dissociated FDB myofibers match findings from dissected EDL myofibers, but SP was up to 14-fold higher in the FDB than EDL. SP was 27% lower in mdx myofibers and 50% less in branches of split mdx fibers compared to the trunk. AP amplitude was not altered in between groups, but this work is ongoing.
Conclusions: Data indicate a reduction in muscle stiffness, increased sarcolemmal deformability and instability in mdx muscle. This approach corroborates the labor-intensive data obtained from single fiber dissection and allows a facile high throughput model. Findings suggest mechanical differences due to altered morphology, despite comparable excitability.

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Mir181A Targets the 3'UTR of MG29, a Muscle-Specific Synaptophysin Family Gene, for Down-Regulation of MG29 Expression in Dystrophic Skeletal Muscle

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MG29 is a muscle-specific member of the synaptophysin family protein that participates in controlling the maturation and development of transverse tubule (TT) structure and the maintenance of intracellular Ca^{2+} signaling in skeletal muscle. Genetic ablation of MG29 leads to defective formation of the TT network in skeletal muscle, which resembles the abnormal TT structure observed in dystrophic muscles. We found that MG29 protein is drastically reduced in mouse models with muscular dystrophy (e.g. mdx and dysferlin-/-), and such reduced MG29 expression and disrupted TT structure were also observed in human patient biopsies with muscular dystrophy. The mg29 gene contains a unique 3' untranslated region (UTR) with potential binding sites for microRNA (miRNA) or RNA-stabilizing factors. Mutagenesis studies revealed that miR-181a could target a region in the 3′UTR that is highly conserved between mouse and human mg29 genes to exhibit posttranscriptional control of MG29 expression in skeletal muscle. Overexpression of anti-sense miR-181a by microinjection into mouse flexor digitorum brevis fibers significantly increased the expression of endogenous MG29. Studies from other investigators have shown that miR-181a was elevated in human patients with muscular dystrophy. These data suggest an intriguing possibility that elevated miR-181a may be a contributing factor for the reduced MG29 expression under dystrophic conditions. Further understanding of the post-transcriptional regulation by microRNA of MG29 expression can provide more insights into the therapeutic targets of muscular dystrophy.

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Calcium Sensitivity after Active Shortening in Rabbit Psoas Fibres

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Introduction It has been shown that isometric force after active shortening of muscles is lower than the purely isometric force performed at the corresponding lengths. This property is termed force depression. Force depression has been observed in isolated muscle preparations and muscles *in situ* and *in vivo* (Rassier et al., *J Applied Physiol* 2004). The origin of force depression is still unknown. It has been suggested that active shortening may affect calcium sensitivity by changing calcium-troponin binding equilibrium leading to decreased force after active shortening (Ekelund and Edman, *Acta Physiol Scand* 1982). Our aim in the present study was to test whether calcium sensitivity was decreased after active shortening.
Methods Experiments were performed using skinned fibres (n=7) isolated from rabbit psoas muscle. Calcium sensitivity was characterized by establishing the force-pCa curves for isometric reference contractions performed at an average sarcomere length of 2.4 μm and active shortening contractions from an average sarcomere length of 3.0 μm to an average sarcomere length of 2.4 μm. pCa_{50} and the coefficient of cooperativity (n_H) were compared between reference and active shortening contractions.
Results and Discussion pCa_{50} for the reference contractions was 5.99 ± 0.02. No change in pCa_{50} was observed in the force depressed state (6.00 ± 0.01). Furthermore, the coefficient of cooperativity did not change after active shortening (3.9 ± 0.3 versus 4.3 ± 0.3 for the reference and force depressed states respectively). These results suggest that calcium sensitivity does not decrease after active shortening and that the binding interactions between troponin and calcium are not altered in the force depressed state.